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A *Plasmodium falciparum* exo-antigen alters erythrocyte membrane deformability

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Here we describe a reduced membrane deformability of human erythrocytes when aspirated into 0.6 μm diameter pores in polycarbonate sieves, after exposure of uninfected cells to spent parasite-culture supernatant. This, taken in concert with a previous observation that intra-erythrocytic development of the parasite *P. falciparum* decreases host localised membrane deformability, may indicate a biological role for such parasite-induced changes in the rheological properties of the erythrocyte.

Spent-parasite culture supernatant; Erythrocyte membrane deformability; *Plasmodium falciparum*

1. INTRODUCTION

Exo-antigens that are released in a stage-specific fashion and bind to uninfected erythrocytes have been identified in the supernatant culture fluids of the human malaria parasite, *Plasmodium falciparum* [1,2]. The role of these and a plethora of other as yet uncharacterised exo-antigens during merozoite invasion has not been defined.

It has been observed that uninfected cells in parasite cultures, when drawn under a coverslip and examined as a wet-mount preparation, assume various morphological forms, ranging from Type I and II echinocytes to stomatocytes, but predominantly normal discocytes [3]. Uninfected cells in fresh culture medium examined in this way form mainly Type III and IV echinocytes [3].

Plasmodium lophurae antigens have been shown capable of distorting the morphology of uninfected host (duck) erythrocytes [4].

Here we examine the effect that *Plasmodium falciparum* exo-antigens have upon the deformability, and thus the invadability [5] of uninfected human erythrocytes, and characterise the heat-sensitivity and binding-affinity of these antigens.

2. MATERIALS AND METHODS

2.1. *Parasites*

Plasmodium falciparum D_{10} (knobby) clone of strain FCQ-27/PNG was maintained in asynchronous culture as described [6], in human type-O erythrocytes at 37°C and 5% haematocrit using a RPMI1640 TES medium supplemented with 10% human serum in an atmosphere of 5% CO_2:5% O_2:90% N_2.

Cells were harvested from cultures of 5–10% parasitaemia at 2000 × *g*, 4°C, for 10 min, and the supernatant reserved. As well, parasitised cells were harvested at schizont stage, and returned to methionine-deficient culture medium with the addition, (30 μCi/ml) of [^{35}S]methionine (Amersham; specific activity > 1000 Ci/mmol). The cells were allowed to grow through one cycle before being harvested. The supernatant was heated at 100°C for 10 min, and centrifuged at 2000 × *g* for 10 min to remove precipitated proteins. Samples from before and after heat-treatment were analysed by SDS-polyacrylamide gel electrophoresis [7] and ^{35}S-labelled protein detected by fluorography.

2.2. *Membrane deformability*

Erythrocyte membrane deformability was measured using the method of Brailsford et al. [8]. The cells at a 20% haematocrit in either phosphate-buffered saline (PBS), fresh parasite culture medium, spent-parasite culture supernatant or concentrated spent-parasite culture supernatant, (before and after heat-treatment) after 1 h incubation at 37°C, were passed over 25 mm diameter polycarbonate sieves containing pores said by the manufacturers (Nucleopore Corp., Pleasanton, CA) to have a mean diameter of 0.6 μm, and measured by us at 0.56 μm. The cells were aspirated, fixed and examined as described [9], using a Philips-505 scanning electron microscope.

The bending stiffness and elastic modulus of the membranes were calculated using equations derived by Brailsford et al. [8].

3. RESULTS

In Fig. 1 the scanning electron micrographs display typical membrane protrusions formed before (A) and after (B) incubation of uninfected erythrocytes in spent-parasite culture supernatant.

Over the pressure range studied, membrane protrusion lengths increased with an increase in the applied pressure difference. The same degree of erythrocyte membrane deformability (i.e. length of protrusion formed) was found irrespective of whether the cells were

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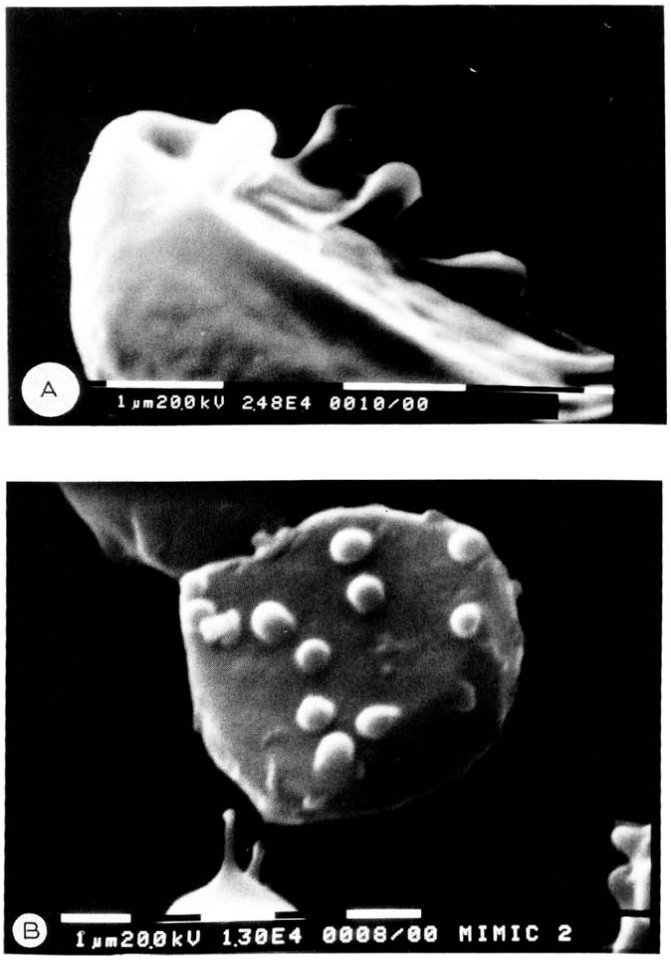

Fig. 1. Scanning electron micrographs of uninfected erythrocytes in (A) PBS, and (B) spent-parasite culture supernatant. Hydrostatic pressure 5.2 cm. Magnification is as indicated on the diagram, with dimensions shown on the horizontal bars.

incubated in PBS (data not shown) or complete parasite culture medium prior to aspiration. However, incubating these same cells in the culture medium after growth of the parasite significantly decreased the localised membrane deformability of the erythrocytes (Fig. 2A). Washing the erythrocytes with PBS after exposure to the spent-parasite culture supernatant, or heating the spent-parasite culture supernatant to 100°C and removing the precipitated proteins, (compare lanes a and b, Fig. 2B), had the effect of restoring the local membrane deformability to the level of the control (Fig. 2A). Even when the heat-treated spent-parasite culture supernatant was freeze-dried and concentrated up to 5-times, there was no significant difference in the erythrocyte deformability compared to the control (data not shown).

The behaviour of the cells incubated in culture medium was similar to results published [8,9] in that the membrane entered the pores at an applied hydrostatic pressure of approximately 10 mm, whereas a pressure of 28 mm was required for cells incubated/aspirated in

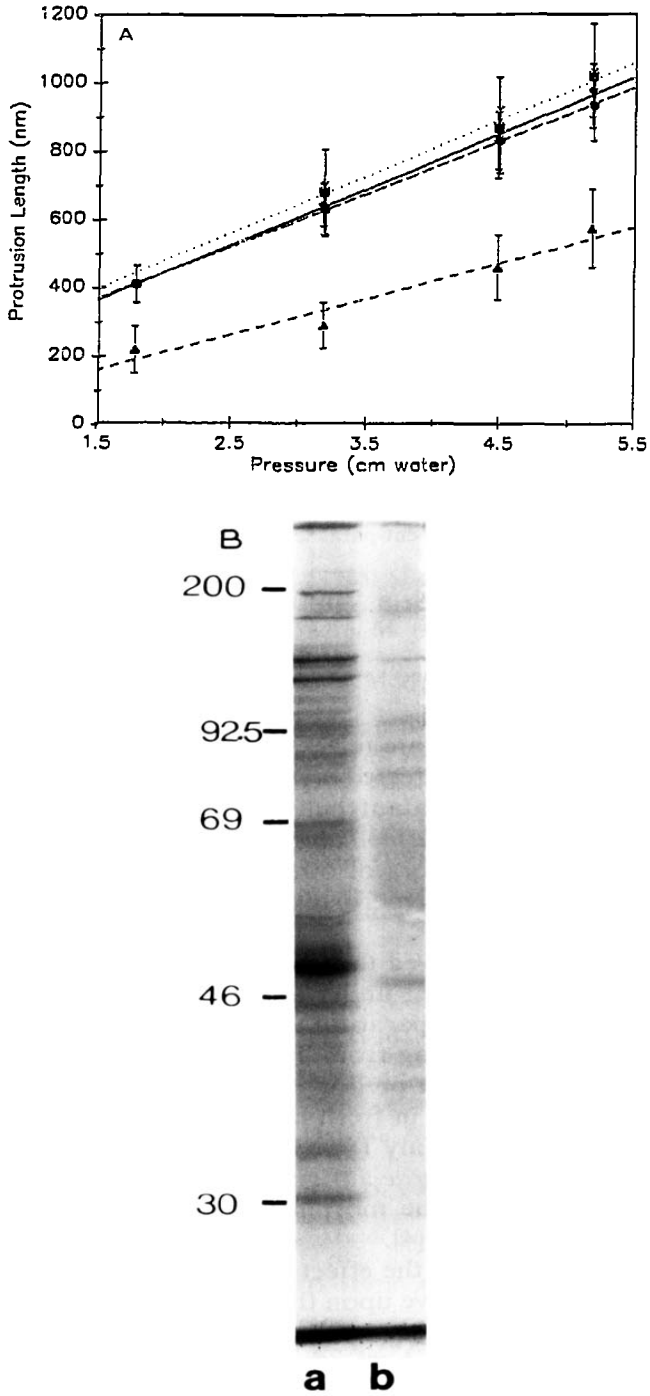

Fig. 2. Erythrocyte deformability. (◆) uninfected cells in culture medium; (▲) uninfected cells in spent-parasite culture supernatant; (●) uninfected cells washed in PBS after incubation in spent-parasite culture supernatant; (■) uninfected cells in heat-treated spent-parasite culture supernatant. Error bars represent standard deviation of 30 data points. (B) Parasite proteins in spent-parasite culture supernatant. Autoradiograph of ^{35}S-labelled spent-parasite culture supernatant, before (a) and after (b) treatment at 100°C for 10 min.

spent-parasite culture supernatant. The minimum pressure required to produce any deformation was used as a measure of the bending modulus and was found to be

Table I

Erythrocyte membrane deformability

Aspiration/ Incubation medium	Bending stiffness (dynes/cm)	Shear modulus (dynes/cm)
Culture medium	4.68×10^{-12}	0.0063
Spent-parasite culture supernatant	1.31×10^{-11}	0.0103

The shear modulus was calculated using a transformation of Eqn. 3 of Brailsford et al. [8] from the slope and intercept of a plot of the alteration in the contraction ratio against applied pressure. These values were: slope 0.934 ± 0.003 (SD) and y-intercept 2.097 ± 0.005 for cells incubated in culture medium; and 1.872 ± 0.005 and 3.066 ± 0.006 respectively for cells incubated in spent-parasite culture supernatant.

3 times greater for cells incubated in the spent-parasite culture supernatant compared to cells incubated in culture medium (Table I). The pressure-dependence of the protrusion length was used as a measure of the membrane shear modulus. The shear modulus was found to be 0.006 dynes/cm for cells incubated in culture medium, a value close to published results [8]. The shear modulus was approximately doubled for cells in spent-parasite culture supernatant (Table I).

Fig. 2B, lanes a and b, show the parasite proteins (since host-derived proteins are not metabolically labelled) present before and after heat-treatment, respectively. The spent-parasite culture supernatant contains a multitude of bands, some of which are not present after heat-treatment.

4. DISCUSSION

During the asexual growth and development of the malarial parasite, a multitude of proteins are released from the infected cells into the media. Certainly many of these may be simply the result of abortive invasions following schizogeny. It is likely, however, that some of these exo-antigens have biological significance. Any one of the number of parasite proteins seen to disappear after heat treatment (Fig. 2B) could be involved in modulating the deformability of uninfected cells. Several recent studies have focussed on a number of malarial antigens and their processed products, isolated from the supernatant or cellular fractions of parasite cultures, that have been characterised as interacting with erythrocyte membrane components. These include gp195, the major merozoite surface glycoprotein [10] that gives rise to 83 and 73 kDa processed products precipitated from culture supernatants [11], and EBA-175 [1,2], a 175 kDa protein released into the culture supernatant during rupture of schizont-infected erythrocytes. The specific binding of EBA-175 to sialic acid-containing determinants of the erythrocyte surface correlates with invasion efficiency.

The heat-sensitive component found to dramatically decrease the deformability of uninfected cells could be one of the mentioned proteins, one of their breakdown products, or a previously uncharacterised *P. falciparum* antigen, acting alone or in conjunction with an erythrocyte or serum protein. The role of this protein, released at a crucial point in the parasites asexual life-cycle could play an important part in host–parasite interactions. It will be important to determine whether uninfected erythrocytes incubated in the presence of this factor resist infection by *P. falciparum*, because by decreasing their deformability, and thus possibly their invadability, intra-erythrocytic parasite numbers would be limited, thus ensuring the survival of the host and ultimately that of the parasite.

In further work carried out to characterise this factor it appears to be the same as that responsible for the altered morphology of uninfected cells examined in wet-mount preparations [3]. Important further work will involve studying the erythrocyte components with which the factor interacts and thus gaining an insight into the mechanism by which the properties of the erythrocyte membrane are modified.

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REFERENCES

[1] Camus, D. and Hadley, T.J. (1985) Science 230, 553–556.
[2] Orlandi, P.A., Kim Lee Sim, B., Chulay, J.D. and Haynes, J.D. (1990) Mol. Biochem. Parasitol. 40, 285–294.
[3] Read, D.G., Bushell, G.R. and Kidson, C. (1990) Parasitology 100, 185–190.
[4] Kilejian, A. (1976) J. Protozool. 23, 272–277.
[5] Pasvol, G., Chasis, J.A., Mohandas, N., Anstee, D.J., Tanner, M.J.A. and Merry, A.H. (1989) Blood 74, 1836–1843.
[6] Trager, W. and Jensen, J.B. (1976) Science 193, 673–675.
[7] Laemmli, U.K. (1970) Nature 227, 680–685.
[8] Brailsford, J.D., Korpman, R.A. and Bull, B.S. (1977) Blood Cells 3, 25–38.
[9] Saul, A., Lamont, G., Sawyer, W.H. and Kidson, C. (1984) J. Cell. Biol. 98, 1348–1354.
[10] Perkins, M.E. (1984) J. Exp. Med. 160, 788–798.
[11] Camus, D., Lyon, J.A., Reaud-jareed, T., Haynes, J.D. and Diggs, C.L. (1987) Mol. Biochem. Parasitol. 26, 21–28.